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Abstract

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Disciplines

Agriculture | Animal Sciences | Meat Science

Comments

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Research Note

Colicin Concentrations Inhibit Growth of *Escherichia coli* O157:H7 In Vitro†

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ABSTRACT

Escherichia coli O157:H7 is a virulent foodborne pathogen that causes severe human illness and inhabits the intestinal tract of food animals. Colicins are antimicrobial proteins produced by *E. coli* strains that inhibit or kill other *E. coli.* In the present study, the efficacy of three pore-forming colicins (E1, N, and A) were quantified in vitro against *E. coli* O157:H7 strains 86-24 and 933. Colicins E1 and N reduced the growth of *E. coli* O157:H7 strains, but the efficacy of each colicin varied among strains. Colicin E1 was more effective against both strains of *E. coli* O157:H7 than colicins A and N and reduced ($P < 0.05$) populations of *E. coli* O157:H7 at concentrations <0.1 μg/ml. These potent antimicrobial proteins may potentially provide an effective and environmentally sound preharvest strategy to reduce *E. coli* O157:H7 in food animals.

Enterohemorrhagic *Escherichia coli* (EHEC) such as *E. coli* O157:H7 annually cause 93,000 illnesses at a cost to the U.S. economy of approximately $1 billion *(7).* Many of these illnesses have been linked to consumption of foods derived from or contaminated by animal products *(4, 21).* Because pathogenic bacteria are commonly found as members of the gastrointestinal population of food animals *(19, 24, 25),* they can be introduced into the abattoir within the feces of cattle or attached to their hide *(5, 6, 8, 26).*

A variety of postslaughter intervention strategies have been implemented in slaughter plants over the past 10 years (e.g., steam treatment, hair removal) that significantly reduce pathogen levels on carcasses *(6, 8).* However, in spite of these innovative interventions, foodborne illnesses linked to meat products still occur far too frequently. Thus, strategies to reduce foodborne pathogens in animals prior to slaughter holds significant promise in reducing human illness *(12).*

Colicins are antimicrobial proteins produced by certain *E. coli* strains that kill or inhibit the growth of other *E. coli* strains *(15, 16, 28).* Colicins have been shown to be effective against *E. coli* O157:H7 strains *(13, 23, 27)*; however, studies examining the efficacy of different colicins have typically yielded qualitative rather than quantitative results. In this study, we quantitatively compared the efficacy of three purified colicins (A, E1, and N) against *E. coli* O157:H7 strains.

MATERIALS AND METHODS

Colicin production and purification. Each colicin was produced from a specific colicin-producing *E. coli* K-12 strain (NC50129-01 containing plasmid pColA-CA31, NC50132-01 containing plasmid pColE1-K53, and NC50145-01 containing plasmid pColN-284) obtained from the National Collection of Type Cultures (Public Health Laboratory Service, London, UK). Cultures were inoculated into Luria-Bertani (LB) broth to an initial optical density at 600 nm (OD_{600}) of approximately 0.1 and incubated in a shaker at 37°C. When the cultures reached $OD_{600} = 0.9$, colicin production was induced by the addition of 0.2 U of mitomycin C per ml of culture (Sigma Chemicals, St. Louis, Mo.). The cell-free supernatant was obtained by centrifugation 5.5 h later and concentrated by ultrafiltration in a stir-cell apparatus (Amicon, Millipore, Bedford, Mass.) across a regenerated cellulose membrane with a 30-kDa cut-off (Millipore). The concentrated sample was then desalted against 10 mM Tris-Cl, pH 8, and purified by ion exchange chromatography. The desalted samples were applied to a column containing Q Sepharose (Amersham Biosciences, Piscataway N.J.), equilibrated with 10 mM Tris-Cl, pH 8.0 (equilibration buffer), and exhaustively washed with the equilibration buffer. The bound protein was eluted with a continuous NaCl gradient on an AKTAprime chromatography system (Amersham Bioscience). The fractions containing the highest concentrations of colicin, determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by Coomasie blue staining, were pooled and concentrated by ultrafiltration. The protein concentrations of these samples were determined in each pooled sample *(20),* and percent colicin was determined by densitometry with the use of a 16-bit megapixel charge-coupled device camera (FluorChem 8800, Alpha Innotech, San Leandro, Calif.) and FluorChem IS800 software (Alpha Innotech).

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† Proprietary or brand names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by the U.S. Department of Agriculture implies no approval of the product or exclusion of others that may be suitable.

Bacterial strains and culture conditions. *E. coli* O157:H7 strains 933 (ATCC 43895) and 86-24 were obtained from the Food and Feed Safety Research Unit culture collection (U.S. Department of Agriculture/Agricultural Research Service, College Station, Tex.); both strains were originally isolated from human hemorrhagic colitis outbreaks. *E. coli* O157:H7 strain 933 was naturally resistant to 25 μg/ml novobiocin and were made resistant to 20 μg/ml nalidixic acid by repeated transfer and selection. *E. coli* O157:H7 86-24 was made resistant to streptomycin (100 μg/ml) by repeated transfer and selection. Differences in growth rates and antibiotic resistance profiles (other than for specifically selected and closely related antibiotics) were not detected between these antibiotic-resistant strains and wild-type parental strains (data not shown).

Escherichia coli O157:H7 strains 933 (ATCC 43895) and 86-24 were anaerobically (90% N_2, 5% H_2, 5% CO_2 atmosphere) incubated at 39°C in anoxic tryptic soy broth (Difco Laboratories, Detroit, Mich.) to ensure colicin activity under anaerobic conditions similar to those within the gastrointestinal tract. Growth rates ($n = 2$) were estimated via measurement of absorbance changes with a Spectronic 20D spectrophotometer (600 nm, Thermo Spectronic Inc., Madison, Wis.); growth rate was calculated with the formula $(\ln OD_2 - \ln OD_1)/\Delta T$. Final optical densities after 24 h of incubation were measured with a Gilford 2600 spectrophotometer (600 nm, 1-cm cuvette). Cultures with optical densities greater than 0.7 OD units were appropriately diluted in 0.9% NaCl.

Quantitative bacterial enumeration. Samples were taken from incubations at 6 and 24 h to determine the effect of colicins on populations of *E. coli* O157:H7. Samples were serially diluted (in 10-fold increments) in phosphate-buffered saline (pH 7.0) and subsequently plated on MacConkey's agar (supplemented with 25 μg/ml novobiocin and 20 μg/ml nalidixic acid for *E. coli* O157:H7 strain 933 or with 100 μg/ml streptomycin for *E. coli* O157:H7 strain 86-24) and incubated at 37°C overnight for direct counting (CFU per milliliter).

Colicin addition. To initially evaluate the effectiveness of these colicins against *E. coli* O157:H7 strains, cultures were inoculated into tryptic soy broth tubes containing equivalent concentrations (4.1 μg/ml) of each of the individual colicins tested (A, E1, and N). To determine the effective range of doses for use in more complex mixed culture and in vivo studies, freshly inoculated tryptic soy broth was added (5 ml) to culture tubes containing concentrations (0 to 40.8 μg of each colicin per ml) of colicin A, colicin E1, and colicin N. The total volume of each colicin addition, including the 0 μg/ml colicin control, was 175 μl; the volume of the colicin dose was made constant by the addition of sterile, anoxic 10 mM Tris, pH 8. To determine the lowest effective dose of colicin E1 against both *E. coli* O157:H7 strains, cultures were grown in the presence of 0, 0.016, 0.032, 0.064, 0.128, 0.255, 0.51, 1.02, 2.04, 4.1, 7.7, 15.4, 28.8, and 40.8 μg of colicin E1 per ml.

Statistical analysis. Experiments were performed in duplicate, and the values presented are means. Students' *t* test was used to determine significance of differences between means.

Chemicals. Unless specifically mentioned, all chemicals were obtained from Sigma Chemical Company.

RESULTS

Effect of colicins on *E. coli* O157:H7. *E. coli* O157:H7 grew rapidly in tryptic soy broth, but the addition of colicins affected growth (Fig. 1a and 1b). Colicin E1 significantly ($P < 0.05$) reduced growth of both *E. coli* O157:H7 strains 933 and 86-24 (Fig. 1a and 1b). Colicin N did not affect the growth rate of *E. coli* O157:H7 strain 933 (Fig. 1a) but did reduce the OD or growth rate of *E. coli* O157:H7 strain 86-24 (Fig. 1b). Colicin A did not affect the OD of either strain of *E. coli* O157:H7. Colicin E1 significantly ($P < 0.05$) reduced the specific growth rate of both strains of *E. coli* O157:H7 examined at low concentrations (Fig. 2a and 2b). Colicin N was nearly as effective as E1 against strain 86-24 but was not ($P > 0.10$) effective against strain 933.

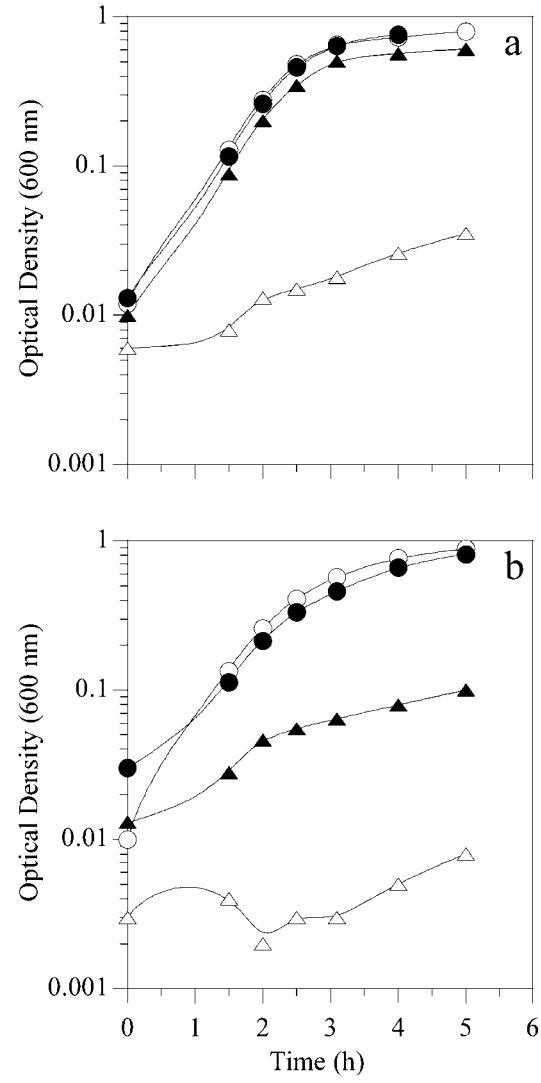

FIGURE 1. *Effect of colicins A, N, and E1 (4.1 μg/ml for each individual colicin) on increase in optical density (600 nm) of* E. coli *O57:H7 strains 933 (a) and 86-24 (b).* ○, *control;* ●, *colicin A–treated;* △, *colicin E1–treated; and* ▲, *colicin N–treated cultures.*

Regardless of the colicin dose used, all *E. coli* O157:H7 cultures eventually grew overnight; therefore, final (24 h) optical densities were not significantly reduced in either strain by any of the colicins (data not shown).

Bacterial populations of both *E. coli* O157:H7 strains were unaffected by colicin A (Fig. 3a and 3b). Treatment with colicin E1 significantly ($P < 0.05$) reduced *E. coli* O157:H7 populations by at least 4 log units for both strains

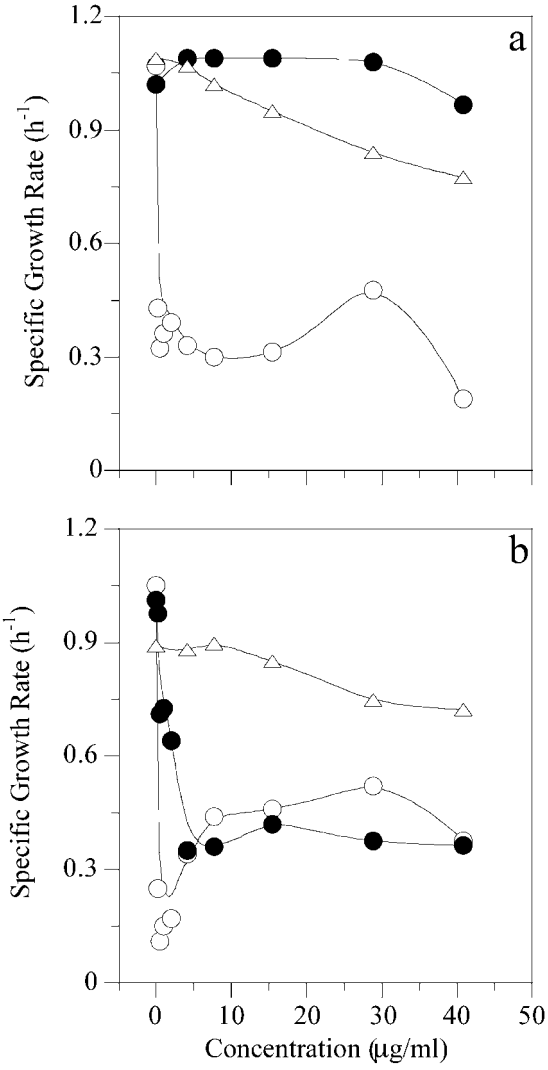

FIGURE 2. *Effect of various concentrations of colicins A, N, and E1 on the maximal specific growth rates (h^{-1}) of* E. coli *O57:H7 strains 933 (a) and 86-24 (b).* △, *colicin A–treated;* ○, *colicin E1–treated; and* ●, *colicin N–treated cultures.*

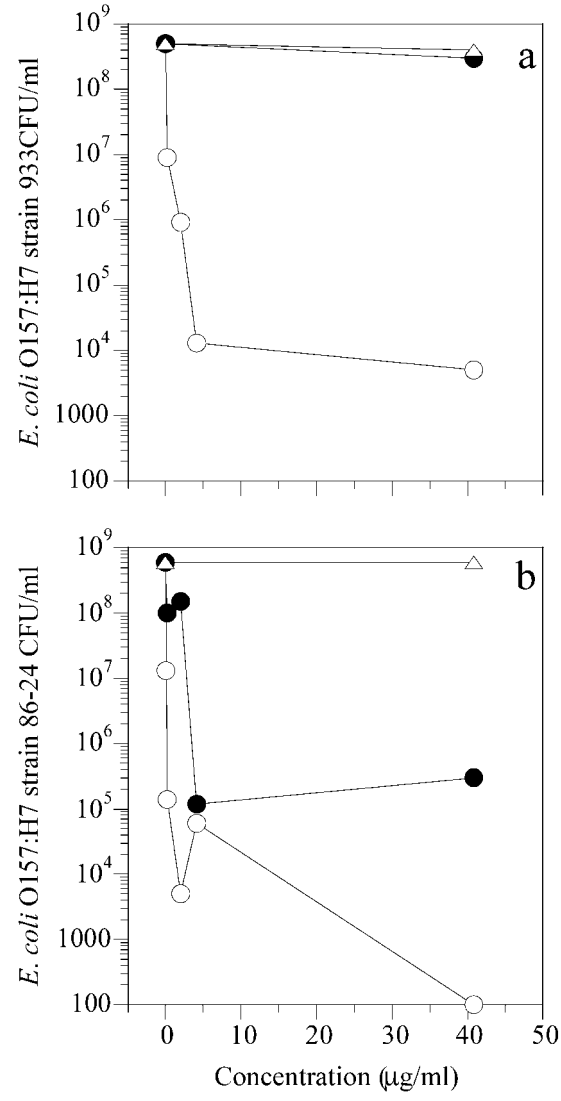

FIGURE 3. *Effect of concentrations of colicins A, N, and E1 on bacterial populations (CFU/ml) of* E. coli *O57:H7 strains 933 (a) and 86-24 (b) after 6 h of incubation.* △, *colicin A–treated;* ○, *colicin E1–treated; and* ●, *colicin N–treated cultures.*

tested, whereas colicin N significantly ($P < 0.05$) reduced populations only for *E. coli* O157:H7 strain 86-24 (Fig. 3b). Because of the sensitivity of both strains of *E. coli* O157:H7 to colicin E1, we examined the efficacy of very low doses against these strains. Colicin E1 reduced ($P < 0.05$) the specific growth rate of both strains of *E. coli* O157:H7 at concentrations below 0.1 μg/ml (Fig. 4). In a negative control, *Salmonella* Typhimurium culture growth rates, final OD, and populations were not affected by any colicin treatment (data not shown).

DISCUSSION

The primary route of infection by *E. coli* O157:H7 in humans is consumption of improperly cooked or handled ground beef *(31),* although serious waterborne outbreaks have occurred *(3, 32).* To reduce exposure of humans to these pathogens, it is therefore logical to conceptualize the use of antibiotics to reduce carriage of foodborne pathogens in the gut *(9).* However this strategy is not likely to be acceptable to the public because of widespread concerns over antibiotic resistance *(1, 33–36).* Therefore alternative antimicrobial strategies that generally or specifically target foodborne pathogens must be developed.

Colicins are small proteins (29 to 75 kDa in size) produced by certain *E. coli* strains that exhibit antimicrobial activity *(15, 16, 28)* by binding to specific outer membrane receptors of sensitive bacteria, translocating across the outer membrane spanning the periplasm, and inserting into the inner membrane *(16–18).* Following insertion into the inner membrane, the pore-forming colicins, such as colicin E1, A, and N, create a voltage-dependent pore that allows ions to flow out of the cytoplasm, destroying the electrochemical gradients and the protonmotive force *(10, 11, 14).* Cellular death results from a loss of K^+, as well as a depletion of intracellular ATP and other phosphorylated intermediates *(16, 30).*

The concept of using colicins as an intervention strategy to kill foodborne pathogens is not new *(23)*; however, this is the first study to quantitatively evaluate the efficacy

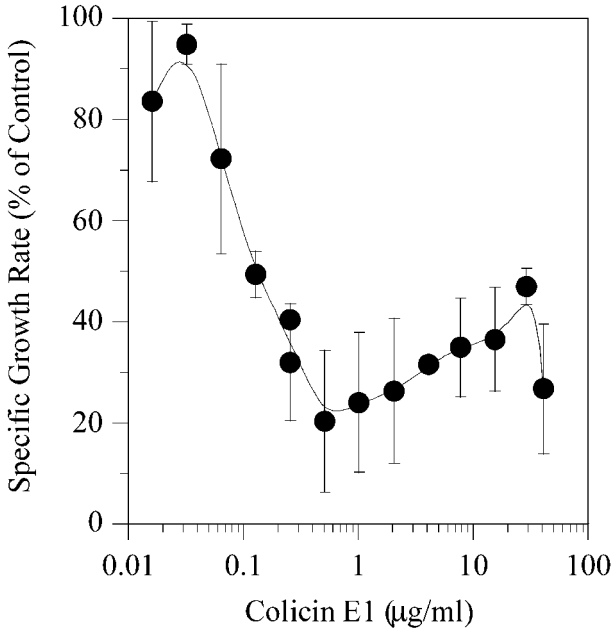

FIGURE 4. *Effect of lowest colicin E1 concentrations (μg/ml) on the maximal specific growth rate (h^{-1}) of* E. coli *O157:H7 strains. Error bars indicate standard deviations.*

of various colicins against *E. coli* O157:H7. In previous studies, colicin activities against several *E. coli* O157:H7 strains were qualitatively determined in agar overlays *(23, 27).* This method of evaluation of colicin activity has been used primarily because of the difficulty in obtaining sufficient quantities of purified colicin proteins. Because of costs associated with the production and purification of these colicins from their native producers, it was unlikely that a quantitative determination of their efficacy would ever be needed for commercial application. However, recent recombinant protein expression work in our laboratories has created a need for this information to be able to compare native versus recombinantly expressed colicins *(29).*

In this study, colicin E1 was the most effective colicin. This result agrees with previous data indicating that colicin E1 displayed antimicrobial activity against several EHEC strains, not just O157:H7 *(13).* Other E-type colicins were found to be suitable candidates as ''biopreservatives'' against *E. coli* O157:H7 *(22).* However, other studies have indicated that the sensitivity of *E. coli* O157:H7 strains to any single colicin can be highly variable *(22, 23, 27).* For example, only 1 of 18 colicins examined inhibited all 540 *E. coli* O157:H7 strains screened *(27).* Because some *E. coli* O157:H7 strains are colicinogenic and produce specific concomitant immunity proteins *(22),* they can be resistant to certain colicins or even a broad category of colicins *(2).* Therefore, simultaneous administration of a mixture of several categories of colicins should be considered as a treatment concept to reduce *E. coli* O157:H7 (and other EHEC) in the gastrointestinal tract of food animals. However, for colicins to be a viable anti–*E. coli* O157:H7 intervention strategy, the proteins must be protected from gastric and intestinal degradation. Technologies exist to target release of compounds in specific locations throughout the gastrointestinal tract; therefore, these techniques should be considered for use in further development and application studies.

In this study, we found that some highly active colicins can be used as a potential method of reducing *E. coli* O157:H7 populations. It is envisioned that colicins could be protected from gastric, intestinal, or both kinds of degradation to serve as an intervention strategy to reduce foodborne pathogenic bacteria in the live animal. Further research is required to elucidate the optimal colicin dosing strategies required to control EHEC carriage in food animals.

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